Brazilian Journal of Development

Calcium and Magnesium Ratio in Alfalfa Cultivation

Proporção Cálcio e Magnésio no Cultivo de Alfafa

DOI:10.34117/bjdv5n12-040

Recebimento dos originais: 07/11/2019
Aceitação para publicação: 04/12/2019

Edna Maria Bonfim-Silva
Department of Agricultural and Environmental Engineering, Institute of Agricultural Sciences and Technology/ Zootechnist
Federal University of Mato Grosso, Rondonópolis, Brazil
E-mail address: embonfim@hotmail.com

Luana Aparecida Menegaz Meneghetti
Department of Agricultural and Environmental Engineering, Institute of Agricultural Sciences and Technology/ Agricultural Engineer graduating
Federal University of Mato Grosso, Rondonópolis, Brazil
E-mail address: embonfim@hotmail.com

Camila Thaiana Rueda da Silva
Department of Agricultural and Environmental Engineering, Institute of Agricultural Sciences and Technology/ Zootechnist
Federal University of Mato Grosso, Rondonópolis, Brazil
E-mail address: embonfim@hotmail.com

André Pereira Freire Ferraz
Department of Agricultural and Environmental Engineering, Institute of Agricultural Sciences and Technology/ Agronomist Engineer
Federal University of Mato Grosso, Rondonópolis, Brazil
E-mail address: embonfim@hotmail.com

Tallys Henrique Bonfim-Silva
Department of Agricultural and Environmental Engineering, Institute of Agricultural Sciences and Technology/ Mechanical Engineer graduating
Federal University of Mato Grosso, Rondonópolis, Brazil
E-mail address: embonfim@hotmail.com

Helon Hébano de Freitas Souza
Department of Agricultural and Environmental Engineering, Institute of Agricultural Sciences and Technology/ Agronomist Engineer
Federal University of Mato Grosso, Rondonópolis, Brazil
E-mail address: embonfim@hotmail.com

Tonny José Araujo da Silva
Department of Agricultural and Environmental Engineering, Institute of Agricultural Sciences and Technology/ Agronomist Engineer
Federal University of Mato Grosso, Rondonópolis, Brazil
E-mail address: embonfim@hotmail.com

Brazilian Journal of Development

Antonio Rodrigues da Silva
Department of Agricultural and Environmental Engineering, Institute of Agricultural Sciences and Technology/ Zootechnist
Federal University of Mato Grosso, Rondonópolis, Brazil
E-mail address: embonfim@hotmail.com

ABSTRACT
The Alfalfa is an important forage for animal feeding, used in breeding of specialized herds due to its high protein content, besides having high palatability and energy value. In this context, the objective of this study was to evaluate the effect of calcium and magnesium ratio in the correction of Oxisol for the cultivation of alfalfa (Medicago sativa L.) cv. Creole. The experiment was conducted in the greenhouse at Federal University of Mato Grosso, Campus Rondonópolis-MT. The experimental design was completely randomized with nine treatments relating about calcium/magnesium nutrients: 0/0; 1/0; 0/1; 3/1; 6/1; 9/1; 12/1; 15/1 and 18/1, with 5 repetitions. The evaluated variables were plant height, number of leaves, number of basal shoots, SPAD index, stem diameter, fresh and shoot dry mass, root dry mass and root volume, soil pHCaCl2 and water use efficiency. The results were submitted to analysis of variance and Scott-knott test ($p \leq 0.05$). Both the first and second cut, plant height was lower (24.76 ± 2.57 and 32.82 ± 13.01 cm, respectively) in absence of fertilizers (0/0) in relation to the other ratios. However, the treatments with Ca and/or Mg did not vary significantly, with a mean of 38.07 ± 2.94 and 51.73 ± 7.43 cm, in the first and second cut, respectively. The shoot dry mass showed similar behavior. Lower mass was observed in absence of Ca/Mg in the first cut (1.12 g DM pot-1) and second cut (1.84 g DM pot-1). In the other relations, it was observed a mean of 2.90 and 4.79 g DM pot-1 between the cuts. Likewise, the production of root was lower in absence of fertilizers (1.66 ± 1.05 g DM pot-1) and the others Ca/Mg ratios did not differ from each other (6.14 ± 1.98 g DM pot-1). The SPAD index there was significant effect only in the first cut between the ratio 0/0 and the others, with a mean of 43.48 ± 2.83 and 48.85 ± 2.19, respectively. In the second cut, SPAD index was 38.47 ± 5.82. Soil pH ranged from 5.87 to 5.31 between the cuts. Alfalfa was low sensitive to calcium and magnesium ratios in soil correction, but responded positively to the plant development with increased base saturation.

Keywords: Medicago sativa L., Calcium/Magnesium ratio, Oxisol.

RESUMO
A alfafa é uma importante forragem para alimentação animal, utilizada na criação de rebanhos especializados devido ao seu alto teor de proteínas, além de apresentar alto palatabilidade e valor energético. Nesse contexto, o objetivo deste estudo foi avaliar o efeito da relação cálcio e magnésio na correção do Latossolo Vermelho para o cultivo de alfafa (Medicago sativa L.) cv. Crioulo. O experimento foi conduzido em casa de vegetação na Universidade Federal de Mato Grosso, Campus Rondonópolis-MT. O delineamento experimental foi inteiramente casualizado com nove tratamentos relacionados aos nutrientes cálcio / magnésio: 0/0; 1/0; 0/1; 3/1; 1/6; 1/9; 1/12; 15/1 e 18/1, com 5 repetições. As variáveis avaliadas foram altura da planta, número de folhas, número de brotações basais, índice SPAD, diâmetro do caule, massa fresca e brotada da massa, massa seca da raiz e volume da raiz, pHCaCl2 do solo e eficiência no uso da água. Os resultados foram submetidos à análise de variância e teste de Scott-knott ($p \leq 0,05$). Tanto no primeiro quanto no segundo corte, a altura das plantas foi menor (24,76 ± 2,57 e 32,82 ± 13,01 cm, respectivamente) na ausência de fertilizantes (0/0) em relação às demais proporções. Entretanto, os tratamentos com Ca e / ou Mg não variaram significativamente, com média de 38,07 ± 2,94 e 51,73 ± 7,43 cm, no primeiro e no segundo corte, respectivamente. A massa seca da parte aérea apresentou comportamento semelhante. Observou-se menor massa na ausência de Ca / Mg no primeiro corte (1,12 g DM pot-1) e no segundo corte (1,84 g DM pot-1). Nas demais relações, observou-se média de 2,90 e 4,79 g de MS pote-1 entre os cortes. Da mesma forma, a produção de raiz foi menor na ausência de fertilizantes (1,66 ± 1,05 g MS pote-

1) e as demais razões Ca / Mg não diferiram entre si (6,14 ± 1,98 g MS pote-1). O índice SPAD teve efeito significativo apenas no primeiro corte entre a razão 0/0 e os demais, com média de 43,48 ± 2,83 e 48,85 ± 2,19, respectivamente. No segundo corte, o índice SPAD foi de 38,47 ± 5,82. O pH do solo variou de 5,87 a 5,31 entre os cortes. A alfafa apresentou baixa sensibilidade às proporções de cálcio e magnésio na correção do solo, mas respondeu positivamente ao desenvolvimento da planta com maior saturação por base.

Palavras-chave: Medicago sativa L., razão cálcio / magnésio, latossolo.

1 INTRODUCTION

The alfalfa (*Medicago sativa L.; Fabaceae*) is recognized as the oldest plant grown exclusively for fodder (Michaud et al., 1988; Prosperi et al., 2014). Due to its occurrence and utilization in various parts of the world, it is one of the most important forage crops (Muller et al., 2003). In addition to geographical comprehensiveness, the alfalfa has a lot of important characteristics such as high yield and forage quality (Lédo et al., 2010).

The culture can be used under grazing or as forage preserved in the form of silage or hay (Borreani & Tabacco, 2006). Under irrigated conditions, alfalfa has forage yield throughout the year (Botrel & Alvim 1997; Bacenetti et al., 2018) with productivity above 20t DM ha^{-1} $year^{-1}$, even in semi-arid regions (Cavero et al., 2016). The successful cultivation of alfalfa may be related to its pivoting root system which can reach more than 6 m deep in the soil and the symbiotic relation with *Rhyzobium* which reduces dependence on nitrogen fertilizers (Michaud et al., 1988).

It is a perennial crop that was introduced in Brazil in the state of Rio Grande do Sul by European immigrants, and after that, its production expanded to other Brazilian states (Ferreira et al., 2004). This forage is a very required food in milk production systems, very important due to its high energy value, productivity and animal acceptability (Ferreira et al., 1999; Rodrigues et al., 2009).

Several factors influence culture productivity, climatic factors, soil, harvest time and especially crop nutritional requirements (Rassini et al., 2008). The mineral nutrition has an essential role in alfalfa cultivation which is highly demanding on nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, boron, among other nutrients (Lanyon & Griffith, 1988). In addition, the relation between soil nutrients can affect crop performance.

Brown & Graham (1978) verified that alfalfa (cv. 'DuPuits', F.C. 40247) may suffer Zn deficiency in alkaline soils and Al toxicity in acidic soils. The authors found that alfalfa developed Cu deficiency symptom when there was liming. Indeed, rise of Ca levels reduce Cu availability (Silva & Williams, 1976; Abreu et al., 2007; Fageria, 2009). It's noteworthy that Cu has an important role for *Fabaceae* as its required in the symbiotic N_2 fixation process (Seliga, 1993).

In another study, Moreira et al. (2008) evaluated different relations between P and Mg in alfalfa cv Florida 77 and they found that increasing P:Mg ratio in the soil until 4:1 (400:100 mg dm^{-}

[3]) provided a linear increase in dry mass production. However, high amounts of P in the soil decreased K absorption. Otherwise, the concomitant addition of K and limestone in soils with low CTC, as occur in the Cerrado, may favor the movement of K to deep layers, may even cause deficiency in plants. This is because Ca and Mg present in dolomitic limestone are more competitive for soil adsorption sites (Ceretta et al., 2007). This can be a problem because K is the most required macronutrient in alfalfa production (Lanyon & Griffith, 1988).

Therefore, it is important studies that seek to investigate the behavior of plants before the relations between different nutrients (Robson & Pitman, 1983). In tropical conditions where soils are generally acidic, the management of Ca and Mg is particularly important in view of the increase of base saturation.

Ca and Mg are generally utilized in the form of limestone to increase soil pH (Troeh, 2007). Soil acidity correction can contribute to increased pasture productivity due to improved soil chemical conditions (Crusciol et al., 2019).

Thus, the objective of this study was to evaluate the effect of calcium and magnesium (Ca/Mg) ratio in the correction of Oxisol for the cultivation of alfalfa (*Medicago sativa*) cv. Creole in protected environment.

2 MATERIAL AND METHODS

The experiment was conducted in the greenhouse at Federal University of Mato Grosso, Campus Rondonópolis, Brazil. The soil utilized was classified as dystrophic Oxisol (EMBRAPA, 2018), which after passed through a 4 mm sieve, was allocated in plastic pots with a capacity of 1.5 kg.

Ca and Mg ratios were calculated initially considering the base saturation increase up to 90%, after it was established how much of the soil CTC (Table 1) should be occupied by Ca, Mg or both. Based on the desire Ca/Mg ratios and the fraction of CTC to be occupied by theses nutrients, it was determined how much Ca and Mg would be required for each treatment.

As Ca and Mg sources were utilized calcium hydroxide ($Ca(OH)_2$) and magnesium hydroxide ($Mg(OH)_2$), which were weighed on a precision balance for treatment composition, represented by the following calcium/magnesium (Ca/Mg) ratios: 0/0, 0/1, 1/0, 3/1, 6/1, 9/1, 12/1, 15/1 e 18/1 (Table 2), with five repetitions, totaling 45 experimental units in a completely randomized design.

Table 1. Chemical and granulometric characteristics of the dystrophic Oxisol, in the 0.02 m deep layer, collected in an area under Cerrado vegetation in the city of Rondonópolis-MT, Brazil.

pH	O.M.	P*	K	S	Ca	Mg	Al	H+Al	SB	CEC
($CaCl_2$)	g kg^{-1}	----- Mg dm^{-3} -----			------------------- Cmolc dm^{-3} -------------------					
4.0	21.2	1.3	33	2.0	0.4	0.2	1.1	5.7	0.7	6.4

V	M	Zn	Mn	Cu	Fe	B	Sand	Silt	Clay
---- % ----		------------ mg dm^{-3} --------------					---------------- g kg^{-1} ----------------		
10.7	61.8	4.0	10.4	0.6	60.0	0.15	445	100	455

*Determined reading in $Melich^{-1}$.

Table 2. *Ca $(OH)_2$ e Mg $(OH)_2$ concentration of each treatment.*

TREATMENT	Ca $(OH)_2$ (g dm^{-3})	Mg $(OH)_2$ (g dm^{-3})
0/0	0	0
0/1	0	2.442
1/0	2.973	0
3/1	2.173	0.544
6/1	2.517	0.273
9/1	2.653	0.165
12/1	2.727	0.106
15/1	2.773	0.070
18/1	2.805	0.045

In each plastic bag (experimental plot) containing 1.5 kg of soil, was added calcium hydroxide, magnesium hydroxide and water, maintaining soil moisture at 80% of pot capacity (Figure 1A).

Figure 1. Plastic bag with 1.5 kg of soil, 60 ml of water and corresponding Ca/Mg treatment (A) and pot with alfalfa seed at sowing time (B).

The soil was homogenized with the hydroxides in plastic bags and they were allocated in the greenhouse for a period of 20 days, period necessary for the soil correction reaction to occur. The pots were arranged on benches at 80 cm height.

The basic fertilization was 150 mg dm^{-3} of potassium (K_2O) using as a source the potassium chloride, divided twice, 60% at sowing and 40% after the first cut; 800 mg dm^{-3} of phosphorus (P_2O_5) in the form of simple superphosphate and micronutrients at the dose of 15 mg dm^{-3} of FTE (Fritted Trace Elements), containing 9% - Zn, 1.8% - B, 0.8% - Cu, 2% - Mn, 3.5% - Fe and 0.1% - Mo), both added at the time of sowing (Bonfim-Silva et al., 2018).

After fertilization the plastic bags were allocated in pots for sowing. Therefore, alfalfa cv Creole seeds were used (Figure 1B). Prior to fertilization soil samples were collected from each treatment for pH analysis and read using a digital pH meter using calcium chloride ($CaCl_{2\ 0.01}$ Mol L^{-1}) solution.

Alfalfa sowing was performed using 20 seeds per pot and after emergence two thinning were realized, the first at six days after emergence (DAE), leaving 10 plants per pot, and the second at 15 DAE keeping three plants per pot (Figure 2).

Figure 2. Alfalfa plants before (A) and after first thinning (B), and before (C) and after second thinning (D).

For nitrogen fertilization was used as a source the urea, divided twice, applied in solution in which 8.25g of urea was dissolved in 1L of water. The same amount of N was added in each treatment with 25 mL syringe, distributing the solution on the ground to prevent damage to the leaves of the plants. The first portion of N was subdivided and applied at 52 DAE and 59 DAE.

The response of the plants to the treatments was evaluated from two cuts: at 46 DAE and 79 DAE. The response variables were:

- Plant height (P_{height}): average height of the three plants per pot obtained with the aid of a graduated ruler, considering the extended plant.
- Stem diameter (SD): average stem diameter of the three plants measured with digital caliper.
- Number of leaves (NL): manual counting;
- Number of basal shoots (NBS): average considering the accounting of all shoots of the three plants;
- SPAD index (SPAD): evaluated with the aid of a portable chlorophyll meter (electronic meter of relative chlorophyll content) in three leaves per pot;
- Fresh and shoot dry mass (DM): after each cut, the collected biomass was weighed in a semi-analytical balance and placed in a forced air circulation oven for 72 h at 65ºC;
- Root dry mass (RDM) and root volume (V_{root}): After the second cut, the roots of each plant were placed on a 4 mm sieve and washed with running water. The roots retained in the sieve were collected and placed in a graduated cylinder with a known volume of water. By the difference in the volume of water displaced in the cylinder, the root volume was determined. The roots were then placed in properly identified paper bags and dried in an oven at 65ºC for 72 h;
- Water use efficiency (WUE): Therefore, it was necessary to determine pot capacity. In this context, the pot mass was obtained with saturated soil (1800g). Thus, during the experiment, each pot was weighed in a semi-analytical balance to determine the depth of water required to reach 1800g. With the sum of the depth of water applied, the water use efficiency was calculated as described in the equation 1:

$$\mathrm{WUE} = \frac{\mathrm{DM}}{\sum \mathrm{DW}} \qquad (1)$$

WUE = water use efficiency; DM = shoot dry mass in each cut; and ∆DW = sum of the depth of water applied.

The results were subjected to analysis of variance and the Scott-Knott grouping test with the aid of SISVAR 5.6 software. Results were considered significant when $p \leq 0.05$.

3 RESULTS AND DISCUSSION

The analysis of variance indicated differences between the evaluated treatments ($p<0.05$). However, in general, the response variables were little influenced by the different Ca/Mg ratios used. The main statistical effects were found between the treatment without Ca or Mg (treatments 0/0) in relation to the other ratios, i.e., in absence of liming (0/0) provided lower performance of the crop in relation to Ca/Mg ratios studied. This behavior may be due to the fact that alfalfa crop is sensitive to acid soils (Rice et al., 1977; Grewal & Williams, 2003), which may have significantly reduced its performance under absence of liming.

Regarding the relations in which Ca and / or Mg were included, in general, there was no significant effect between liming levels (all treatments were elevated to 90% of base saturation). This may be related to the imbalance created between different applied nutrient doses. According to Domingues et al. (2016), calcium is involved in complex reactions with other chemical elements present in the soil solution, and it has been difficult to estimate the soluble amounts available for absorption by plants. In another study, Oliveira et al. (2000) evaluated different levels of Mg in the soil (0.8 and 16 mmolc dm^{-3}) cultivated with common bean and found that Mg absorption was proportional to the levels used, but Ca absorption was higher when plants were developed in 8 mmolc cm^{-3} of Mg.

When Ca/Mg is added to the soil in equal proportions, this effect may be reduced, or not occur. In this context, Moreira et al. (2011) evaluated doses of dolomitic limestone (0; 3.8; 6.6 and 10.3 t ha^{-1}) in alfalfa cv. 'Creole', in also two cuts and didn’t find significant difference in the Ca/Mg ratio of the soil.

4 PLANT HEIGHT

Plant height is a very important variable because it has a direct relation with the availability of nutrients in the soil for crop development. Regarding germination and initial growth, it was found that the plants subjected to treatment 0/0 (Ca/Mg) took longer to develop compared to the other ratios, i.e., these plants showed greater sensitivity to absence of liming. After the first cut, it was observed that the 0/0 ratio provided lower culture growth (Figure 3A and B).

Figure 3. Alfalfa plant growth curve at 46 days after emergence (A) and 79 days after emergence (B) as a function of treatments with calcium/magnesium ratios 0/0, 0/1, 1/0, 3/1, 6/1, 9/1, 12/1, 15/1 and 18/1, respectively, in the Oxisol.

Regarding the evaluations, there was significance in both cuts realized for plant height (Figure 4). In the first evaluation, at 46 DAE, alfalfa height was influenced by the omission of calcium and magnesium and the plants showed the lowest growth (24.76 cm). The other relations studied didn’t differ statistically. In the second evaluation, at 79 DAE, it was observed again that the 0/0 ratio was the most influenced by the omission of soil nutrients and in one of the repetitions one plant didn’t survive the cuts. The other relations didn’t obtain statistical difference too. Guimarães Junior (2013) evaluated the Ca/Mg ratio in different forage plants and also verified that plant height was not influenced by Ca/Mg ratios.

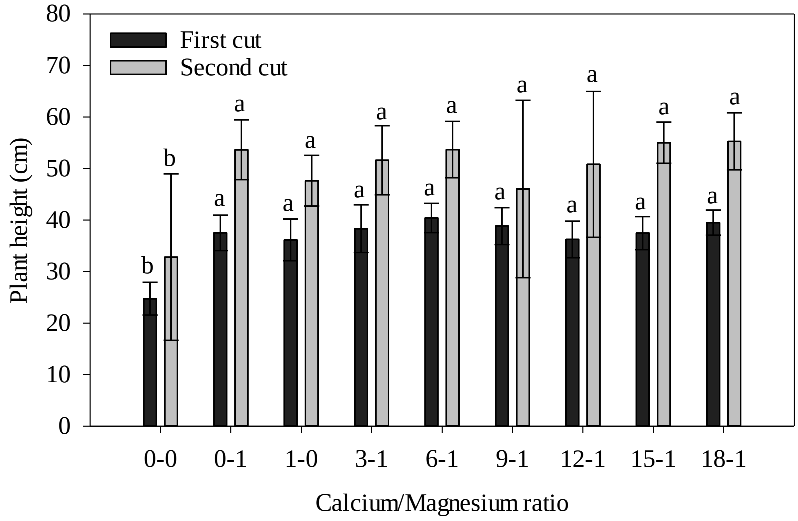

Figure 4. Plant height (cm) of alfalfa at 46 (First cut) and 79 (second cut) days after emergence under calcium/magnesium ratios. For the same cut, the absence of letters on the vertical bars doesn't indicate significant differences between treatments. Vertical bars are the confidence interval for the mean (α=0.05).

5 NUMBER OF LEAVES

In the first cut, the smallest number of leaves was observed in the 0/0 ratio (13.80 ± 4.66 leaves), while in the 18/1 ratio the number of leaves was higher (32.80 ± 2.39 leaves) (Figure 5). In the second cut, under absence of liming (0/0), the smallest number of leaves was observed, approximately 33 leaves, and the highest number of leaves was observed in the 3/1 ratio, approximately 70 leaves. These results are in accordance with those reported in the literature, which suggest the Ca/Mg ratio of 3/1 to 7/1 as the most suitable range for most crops (Eckert, 1987; Kopittke & Menzies, 2007).

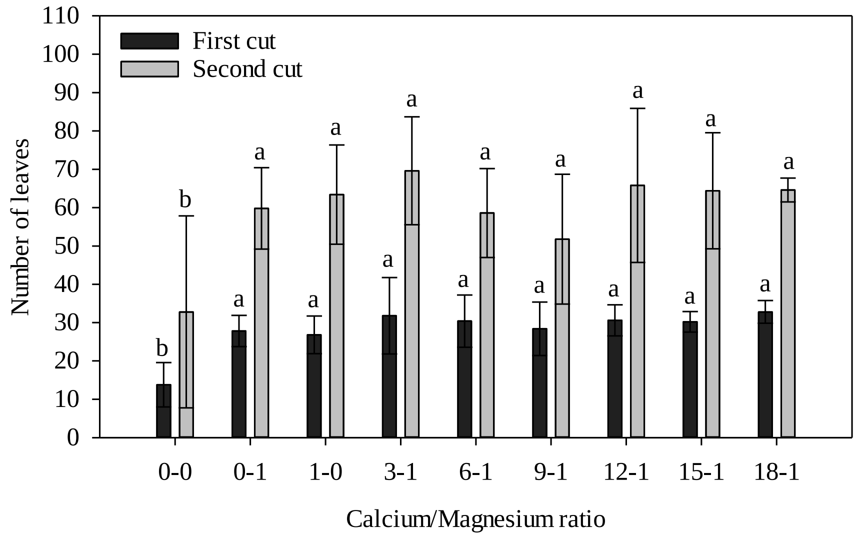

Figure 5. Number of leaves in alfalfa at 46 (First cut) and 79 (second cut) days after emergence under calcium/magnesium ratios. For the same cut, the absence of letters on the vertical bars doesn't indicate significant differences between treatments. Vertical bars are the confidence interval for the mean (α=0.05).

6 STEM DIAMETER

There was no significant effect for the first stem diameter evaluation (Figure 6). These results corroborate those obtained by Bonfim-Silva et al. (2018) who observed that the increase of liming didn't influence the stem diameter of alfalfa. The authors, however, emphasized the importance of calcium in the development of the plant stem.

In the second evaluation, there was a significant effect for the stem diameter (Figure 6). Statistically the 0/0 ratio and 3/1 differ from the others. The highest diameter was found in the 0/1 ratio with 2.26 mm, and the smallest value was found in 0/0, with 1.51 mm.

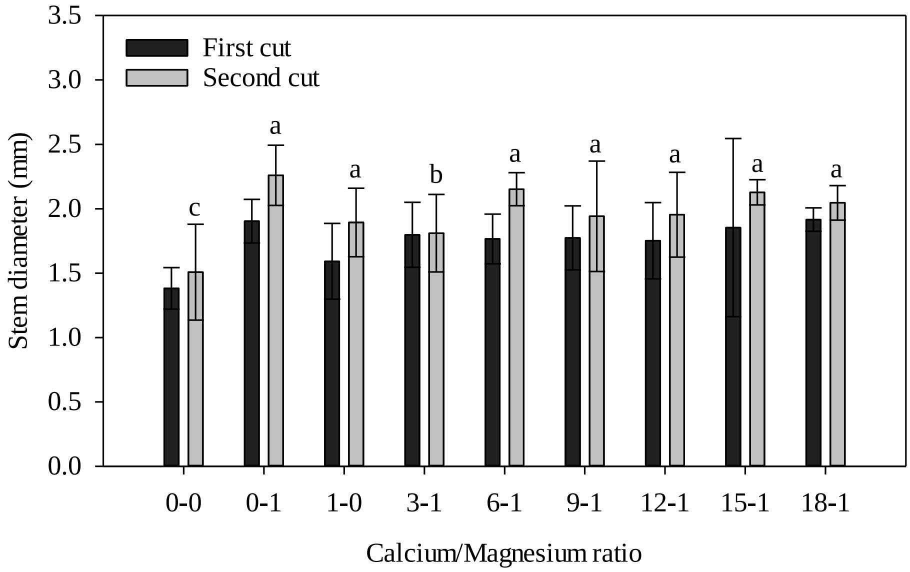

Figure 6. Stem diameter (mm) of alfalfa at 46 (First cut) and 79 (second cut) days after emergence under calcium/magnesium ratios. For the same cut, the absence of letters on the vertical bars doesn't indicate significant differences between treatments. Vertical bars are the confidence interval for the mean (α=0.05).

7 NUMBER OF BASAL SHOOTS

It was observed in the first evaluation at 46 DAE significant difference for the number of basal shoots in the calcium and magnesium relations. Treatments 0/0, 0/1, 1/0, 9/1 and 12/1 didn't differ statistically, as well as the ratios 3/1, 6/1, 15/1 and 18/1 that showed no statistical difference between them (Figure 7).

The highest number of basal shoots (2.00 ± 0.71) was found in the 15/1 treatment at 46 DAE. The Ca/Mg ratio that produced the smallest number of basal shoots was 0/0 and 0/1 with 1.00 ± 0.00

and 1.00 ± 0.71 units, respectively. In the second cut, 79 DAE, there was no significant effect between Ca/Mg ratios evaluated.

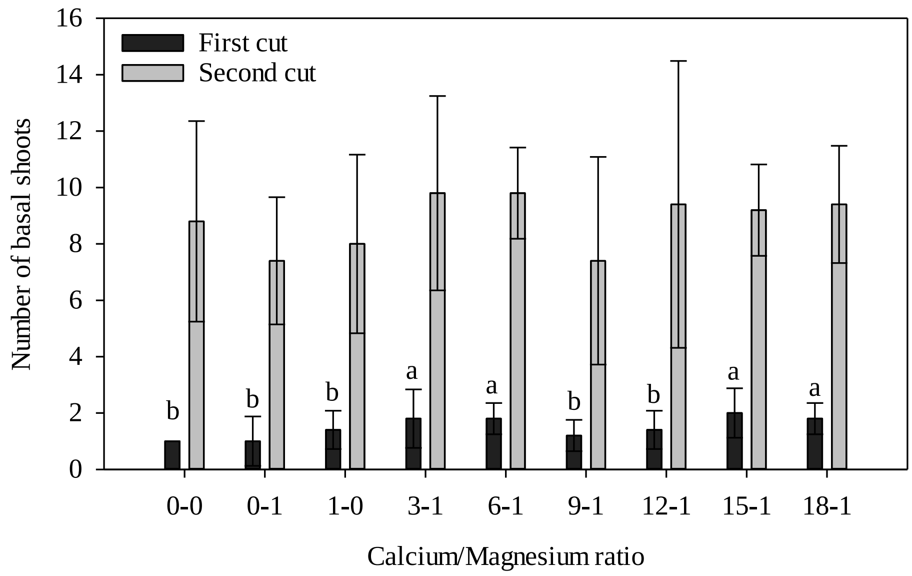

Figure 7. Number of basal shoots of alfalfa at 46 (First cut) and 79 (second cut) days after emergence under calcium/magnesium ratios. For the same cut, the absence of letters on the vertical bars doesn't indicate significant differences between treatments. Vertical bars are the confidence interval for the mean (α=0.05).

8 SHOOT DRY MASS

There was a significant effect for the shoot dry mass in both cuts (Figure 8). In the first cut, the omission of calcium and magnesium caused lower production of shoot dry mass with 1.12 g pot^{-1} and the 18/1 ratio presented the highest production with 3.28 g pot^{-1}, however, only the 0/0 ratio differs statistically from the others.

In the second evaluation, the lowest and highest dry mass production was observed in the ratio 0/0 (1.84 g pot^{-1}) and 3/1 (5.56 g pot^{-1}), respectively, and there was no significant difference between Ca and/or Mg inclusion ratios. These results corroborate those of Moreira et al. (2000) who in their research of calcium and magnesium ratios about alfalfa micronutrients found that Ca/Mg ratios didn't significantly influence in higher dry mass production, but alfalfa responded to the increased application of correctives.

Oliveira & Parra (2003) evaluated bean plant submitted to Ca/Mg ratios and also didn't observe difference in bean production in relation to the different treatments of Ca/Mg. However, in relation to other crops, such as corn, there may be a decrease in productivity with increasing ratios of Ca/Mg. Medeiros et al. (2008) studied Ca/Mg ratios in corn and affirmed that using a high Ca and Mg corrective reduced the absorption of magnesium and potassium by the plant. Thus, there was an increase in calcium level and consequently a nutritional imbalance affecting the physical and productive characteristics of the plant. Guimarães Junior (2013) in his studies about forages subjected to different Ca/Mg ratios didn't find behavior indicating a tendency of responses for forages to increase or reduction of treatments (Ca/Mg) as a function of plant development and productivity.

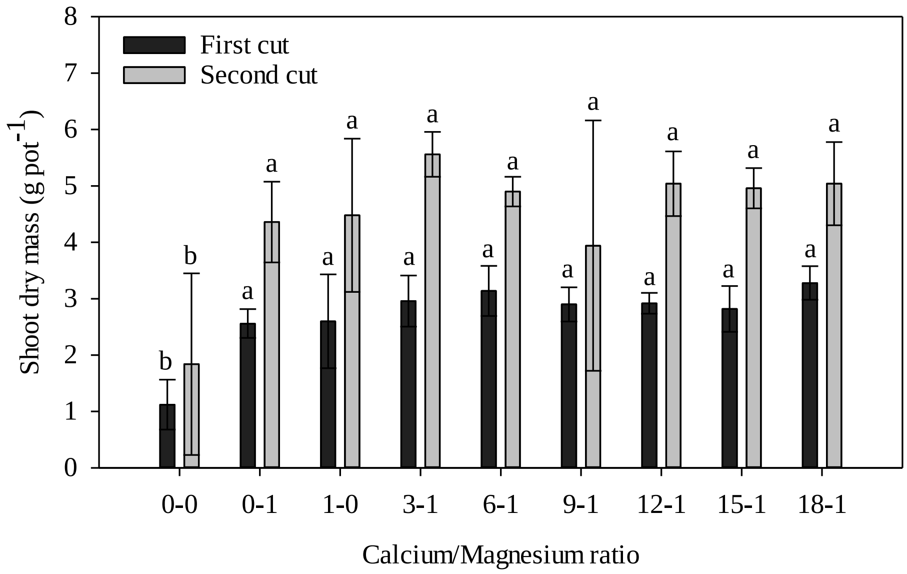

Figure 8. Shoot dry mass of alfalfa at 46 (First cut) and 79 (second cut) days after emergence under calcium/magnesium ratios. For the same cut, the absence of letters on the vertical bars doesn't indicate significant differences between treatments. Vertical bars are the confidence interval for the mean (α=0.05).

9 WATER USE EFFICIENCY

There was a significant effect on water use efficiency in both cuts (46 and 79 DAE) (Figure 9). In both the first cut and second cut, the absence of liming (0/0) caused the lowest water use efficiency (0.48 ± 0.12 and 0.66 ± 0.36 g DM mm^{-1}, respectively). The 0/0 treatment differed from all the others, but among the other Ca/Mg inclusion ratios there was no significant difference which presented an average of 0.95 ± 0.10 and 1.29 ± 0.20 g DM mm^{-1} for the first cut and second cut respectively.

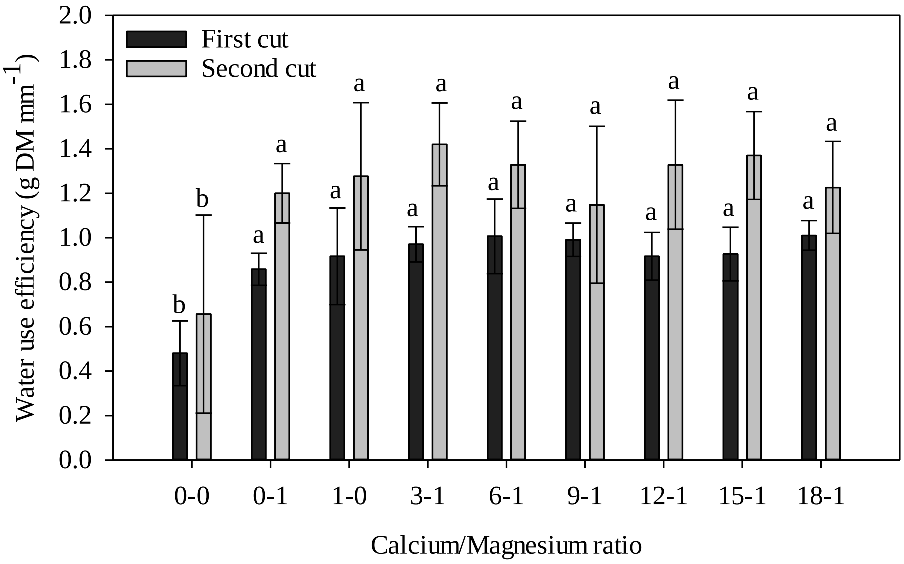

Figure 9. Water use efficiency of alfalfa at 46 (First cut) and 79 (second cut) days after emergence under calcium/magnesium ratios. For the same cut, the absence of letters on the vertical bars doesn't indicate significant differences between treatments. Vertical bars are the confidence interval for the mean (α=0.05).

10 ROOT VOLUME AND ROOT DRY MASS

Root volume was influenced by Ca/Mg ratios (Figure 10A). Analyzing the treatments, the 0/0 ratio obtained the lowest root volume (18.6 mL) differing from the other treatments. The 9/1 ratio treatment had the second lowest root volume, 31.6 mL.

There was a significant effect for root dry mass on Ca / Mg ratios (Figure 10B). In treatment 0/0 (omission of Ca and Mg) the lowest root dry mass was observed (1.66 g). Moreover, it was the only treatment to differ from the others, which on average were responsible for a production of 6.14 ± 1.98 g (Figure 10B), i.e., the alfalfa root system was not influenced by the different ratios of calcium and magnesium. In this context, Bonfim-Silva et al. (2018) observed that low calcium availability affects alfalfa root development and growth.

Brazilian Journal of Development

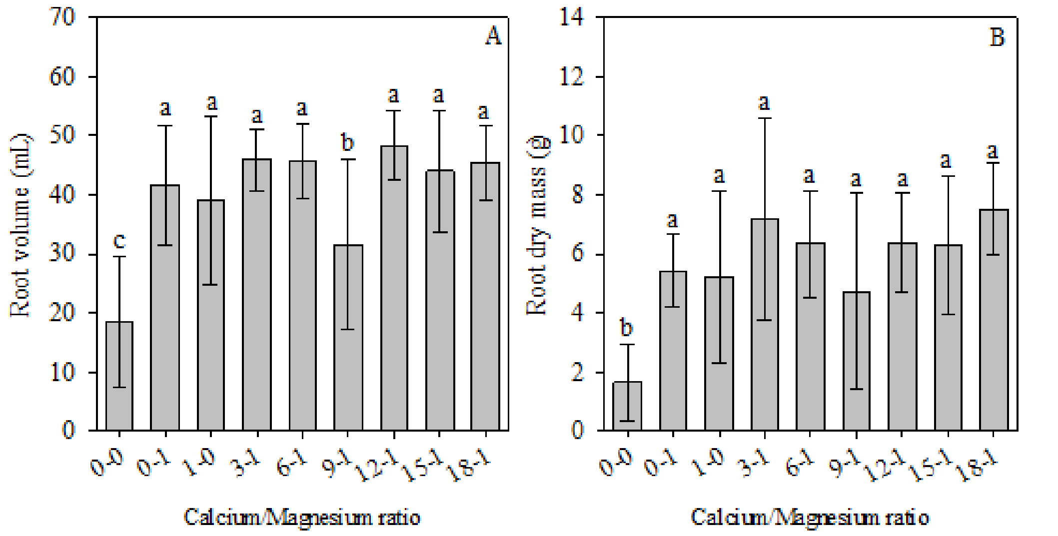

Figure 10. Volume (A) and root dry mass (B) of alfalfa plants at 79 days after emergence as a function of calcium/magnesium ratios. Equal letters do not indicate statistically significant differences between treatments ($p \leq 0.05$). Vertical bars are the confidence interval for the mean ($\alpha = 0.05$).

Omission of calcium can affect negatively the root growth (Domingues et al., 2016). For the formation of nodules, liming to the plant is extremely important. After nodulation occurs, the plant symbiotically fixes atmospheric N_2 and can develop at relatively low Ca and Mg concentrations (Grewal & Williams, 2003). In this study, it was possible to visualize the difference in liming omission treatment compared to the others, in which the 0/0 ratio caused the lowest root volume (Figure 11), as evidenced by the statistical analysis.

Figure 11. Roots of alfalfa cv. Creole at 79 days after emergence (final cut). Treatments: 0/0, 0/1, 1/0, 3/1, 6/1, 9/1, 12/1, 15/1 and 18/1, respectively.

11 SPAD INDEX AND SOIL PH

For the SPAD index there was a significant effect only at the first evaluation (46 days after emergence) which allowed verifying the treatment 0/0 with lower chlorophyll index (43.48). In addition, it differed from other treatments (Figure 12A). The other relations didn't differ statistically, in other words, didn't interfere in the increase of chlorophyll index.

It is emphasized that magnesium is very important to the plant. It is an important enzyme activator that contributes critically to the photosynthesis process, because it is part of the chlorophyll molecules and to the subsequent transport of photoassimilates in the plant (Tränkner et al., 2018). This explains the lowest chlorophyll index in the 0/0 ratio (omission of calcium and magnesium). Liming is used to correct the soil and to provide calcium and magnesium. In study developed by Bonfim-Silva et al. (2018) demonstrated that nutrients availability was pH dependent. At very high pH values there is decreased availability. Malavolta (1989) also affirmed that limestone is essential for very acid soils as it allows the effect of mineral fertilization.

Statistically the pH was significant in the two alfalfa evaluations (at 46 and 79 days after emergence). Thus, it was observed that in the first and second evaluation (Figure 12B) even with liming, the pH in the second cut decreased, except for the 0/0 treatment, in other words, the soil became more acid. The results corroborate with Bonfim-Silva et al. (2018) who observed reduction in pH through the consecutive cuts of alfalfa, in which plants absorbed and exported Ca and Mg from the soil. This is because as alfalfa absorbs Ca^{2+} and Mg^{2+} the plants possibly released from the roots, H^+ ions in the soil solution contributing with the acidification of the medium.

In the first evaluation of alfalfa, 46 days after emergence, it was observed that the treatment 0/0 differs from the others, presenting the lowest soil pH (pH 3.86). Treatment 0/1 also differs statistically, being the second most acidic (pH 5.49) (Figure 12B).

In the second evaluation, at 76 days after emergence, it was observed that the Ca/Mg ratio of 0/0 is statistically different from the others, obtaining the lowest soil pH (4.6) even the increased soil pH during the experiment (Figure 12B).

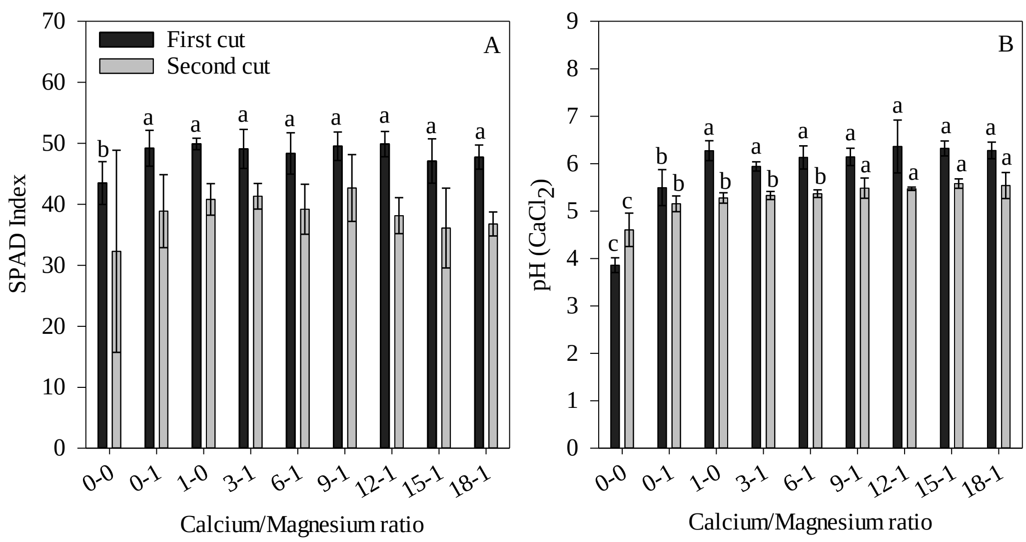

Figure 12. SPAD index (A) and soil pH_{CaCl2} (B) of alfalfa grown under different calcium and magnesium ratios, at 46 and 79 days after emergence. The absence of letters on the vertical bars doesn't indicate significant differences between treatments, in each cut ($p \leq 0.05$). Vertical bars are the confidence interval for the mean ($\alpha = 0.05$).

12 PEARSON (R)* SIMPLE CORRELATION BETWEEN THE RESPONSE VARIABLES OF ALFALFA PLANTS

Regarding the degree of association between the response variables evaluated, there was a significant correlation between most variables (Table 3). It is worth mentioning the effect of soil pH on the morphological and productive characteristics of plants.

Table 3. Pearson (r)* simple correlation between the response variables of alfalfa plants grown at different ratios of calcium and magnesium. Rondonópolis, MT.

	NL	SD	P_{Height}	NBS	SPAD	DM	pH	WUE	Vroot
SD	0.32								
P_{Height}	0.73	0.57							
NBS	0.36	-0.05	0.00						
SPAD	0.42	0.30	0.39	-0.13					
DM	0.84	0.48	0.88	0.24	0.44				
pH	0.65	0.42	0.71	0.06	0.47	0.77			
WUE	0.79	0.43	0.83	0.17	0.43	0.93	0.79		
Vroot	0.70	0.33	0.76	0.22	0.34	0.85	0.60	0.68	
RDM	0.58	0.30	0.67	0.30	0.31	0.77	0.56	0.62	0.79

Coefficients in bold indicate significant correlation ($p \leq 0.05$).
*analysis performed with the average of the two cuts for each variable, except for the volume and root dry mass evaluated in the last cut.

In general, the pH was positively related to increments in the other studied characteristics except for the number of basal shoots, highlighting the shoot dry mass and root production and the SPAD index, which in the final analysis are the most important variables from an economic point of view. Soil correction in cultivation of 14 alfalfa cultivars under liming also resulted in a positive correlation between (Ca and Mg) application and root biomass and shoot length (Adhikari &

Missaoui, 2017). In this context, soil pH management can provide considerable gains of biomass production (quantitative forage factor) and increase of SPAD index which is related to leave chlorophyll content, i.e., forage quality.

Alfalfa is a very demanding crop as far as soil fertility. Adequate nutrient supply is essential for increased forage production and quality maintenance (Adhikari & Missaoui, 2017; Gu et al., 2018).

13 CONCLUSION

Alfalfa was low sensitive to calcium and magnesium ratios in soil correction, but there was a positive response in plant development with increasing base saturation with both Ca and Mg;
In the second alfalfa growth, at the time of the second cut, the soil pH decreased due to the absorption of cations by plants;
The alfalfa forage production and quality were positively influenced by soil pH increase.

REFERENCES

ABREU, C. A.; LOPES, A. S.; SANTOS, G. C. G. Micronutrientes. In: NOVAIS, R. B.; ALVAREZ V., V. H.; BARROS, N. F.; FONTES, R. L. F.; CANTARUTTI, R. B.; NEVES, J. C. L. (Eds.). Fertilidade do Solo. 1. ed. Viçosa, MG: Sociedade Brasileira de Ciência do Solo, 2007. p.645-736.

ADHIKARI, L.; MISSAOUI, A. M. Nodulation response to molybdenum supplementation in alfalfa and its correlation with root and shoot growth in low pH soil. Journal of Plant Nutrition, v.40, n.16, p.2290-2302, Doi: https://doi.org/10.1080/01904167.2016.1264601

BACENETTI, J.; LOVARELLI, D.; TEDESCO, D.; PRETOLANI, R.; FERRANTE, V. Environmental impact assessment of alfalfa (*Medicago sativa* L.) hay production. Science of The Total Environment, v.635, 2018, p.551-558. Doi: https://doi.org/10.1016/j.scitotenv.2018.04.161

BONFIM-SILVA, E. M.; SILVA, C. T. R.; CASTAÑON, T. H. F. M.; FENNER, W.; DAMASCENO, A. P. A. B. Influence of base saturation on growth and yield parameters of alfalfa cultivated in soil of Brazilian Cerrado. Journal of Experimental Agriculture International, v.27, n.4, p.1-10, 2018.

BORREANI, G.; TABACCO, E. The effect of a baler chopping system of fermentation and losses of wrapped big bales of alfalfa. Agronomy Journal, v.98, p.1-7, 2006. Doi: https://doi.org/10.2134/agronj2004.0134

BOTREL, M. A.; ALVIM, M. J. Avaliação de cultivares de alfafa na Zona da Mata de Minas Gerais. Pesquisa Agropecuária Brasileira, v.32, n.9, p.971-975, 1997. https://ainfo.cnptia.embrapa.br/digital/bitstream/item/108678/1/AVALIACAO-DE-CULTIVARES.pdf

BROWN, J. C.; GRAHAM, J. H. Requirements and tolerance to elements by alfalfa. Agronomy Journal, v.70, n.3, p. 367-373, 1978. Doi: https://doi.org/10.2134/agronj1978.00021962007000030002x

CAVERO, J.; FACI, J. M.; MARTÍNEZ-COB, A. Relevance of sprinkler irrigation time of the day on alfalfa forage production. **Agricultural Water Management**, v.178, p.304-313, 2016. Doi: https://doi.org/10.1016/j.agwat.2016.10.008

CERETTA, C. A.; SILVA, L. S.; PAVINATO, A. Manejo da adubação. In: NOVAIS, R. B.; ALVAREZ V., V. H.; BARROS, N. F.; FONTES, R. L. F.; CANTARUTTI, R. B.; NEVES, J. C. L. (Eds.). Fertilidade do Solo. 1. ed. Viçosa, MG: Sociedade Brasileira de Ciência do Solo, 2007. p.851-872.

CRUSCIOL, C. A. C.; MARQUES, R. R.; CARMEIS FILHO, A. C. A.; SORATTO, R. P.; COSTA, C. H. M.; FERRARI NETO, J.; CASTRO, G. S. A.; PARIZ, C. M.; CASTILHOS, A. M.; FRANZLUEBBERS, A. J. Lime and gypsum combination improves crop and forage yields and estimated meat production and revenue in a variable charge tropical soil. Nutrient Cycling in Agroecosystems, p.1-26, 2019. Doi: https://doi.org/10.1007/s10705-019-10017-0

DOMINGUES, L. S.; RIBEIRO, N. D.; ANDRIOLO, J. L.; POSSOBOM, M. T. D. F.; ZEMOLIN, A. E. M. Growth, grain yield and calcium, potassium and magnesium accumulation in common bean plants as related to calcium nutrition. Acta Scientiarum. Agronomy, v.38, n.2, p.207-217, 2016. Doi: https://doi.org/10.4025/actasciagron.v38i2.27757

ECKERT, D. J. Soil test interpretations: basic cation saturation ratios and sufficiency levels. In: BROWN, J. R. (Ed.). Soil Testing: Sampling, Correlation, Calibration, and Interpretation. Madison, WI: Soil Science Society of America, SSSA Special Publication 21, 1987. p.53-64. Doi: http://dx.doi.org/10.2136/sssaspecpub21.c6

EMBRAPA. Sistema brasileiro de classificação de solos. 5. ed., rev. e ampl. − Brasília, DF: Embrapa, 2018 (formato epub). https://www.embrapa.br/solos/busca-de-publicacoes/-/publicacao/1094003/sistema-brasileiro-de-classificacao-de-solos

FAGERIA, N. K. The use of nutrients in crop plants. Boca Raton, CRC Press, 2009. 430p.

FERREIRA, R. P.; BOTREL, M. A.; RUGGIERI, A. N.; PEREIRA, A. V.; COELHO, A. D. F.; LÉDO, F. J. S.; CRUZ, C. D. Adaptabilidade e estabilidade de cultivares de alfafa em relação a diferentes épocas de corte. Ciência Rural, v.34, n.1, p. 265-269, 2004. Doi: http://dx.doi.org/10.1590/S0103-84782004000100041

FERREIRA, R. P.; BOTREL, M. A.; PEREIRA, A. V.; CRUZ, C. D. Avaliação de cultivares de alfafa e estimativas de repetibilidade de caracteres forrageiros. Pesquisa Agropecuária Brasileira, v.34, p.995-1002, 1999. Doi: http://dx.doi.org/10.1590/S0100-204X1999000600010

GREWAL, H. S.; WILLIAMS, R. Liming and cultivars affect root growth, nodulation, leaf to stem ratio, herbage yield, and elemental composition of alfalfa on an acid soil. Journal of Plant Nutrition, v.26, n.8, p.1683-1696, 2003. Doi: https://doi.org/10.1081/PLN-120022381

GU, Y. J.; HAN, C. L.; FAN, J. W.; SHI, X. P.; KONG, M.; SHI, X. Y.; SIDDIQUE, K. H. M.; ZHAO, Y. Y.; LI, F. M. Alfalfa forage yield, soil water and P availability in response to plastic film mulch and P fertilization in a semiarid environment. Field Crops Research, v.215, p.94-103, 2018. Doi: https://doi.org/10.1016/j.fcr.2017.10.010

GUIMARÃES JÚNIOR, M. P. A.; SANTOS, A. C. dos; ARAÚJO, A. S.; OLIVEIRA, L. B. T. de; RODRIGUES, M. O. D.; MARTINS, A. D. Relação Ca:Mg do corretivo da acidez do solo e as características agronômicas de plantas forrageiras. Revista Brasileira de Saúde e Produção Animal, v.14, n.3, p.460-471, 2013.

KOPITTKE, P. M.; MENZIES, N. W. A review of the use of the basic cation saturation ratio and the "ideal" soil. Soil Science Society of America Journal, v.71, n.2, p.259-265, 2007. Doi: https://doi.org/10.2136/sssaj2006.0186

LANYON, L. E.; GRIFFITH, W. K. Nutrition and fertilizer use. In: HANSON, A. A.; BARNES, D. K.; HILL, R. R. (Eds.). Alfalfa and Alfalfa Improvement. Agronomy Monograph 29. Madison, WI: ASA-CSSA-SSSA, 1988. Doi: https://doi.org/10.2134/agronmonogr29.c10

LÉDO, F. J. S.; OLIVEIRA, P. P. A.; PEREIRA, A. V. *Medicago sativa*. In: FONSECA, D. M.; MARTUSCELLO, J. A. (Eds.). Plantas forrageiras. Viçosa, MG: Ed. UFV, 2010. p.341-365.

MALAVOLTA, Eurípedes. ABC da adubação. 5ª ed. São Paulo: Ed. Agronômica Ceres, 1989.

MEDEIROS, J. C.; ALBUQUERQUE, J. A.; MAFRA, A. L.; ROSA, J. D.; GATIBONE, L. C. Relação cálcio:magnésio do corretivo da acidez do solo na nutrição e no desenvolvimento inicial de plantas de milho em um Cambissolo Húmico Álico. Semina: Ciências Agrárias, v.29, n.4, p.799-806, 2008. Doi: http://dx.doi.org/10.5433/1679-0359.2008v29n4p799

MICHAUD, R.; LEHMAN, W. F.; RUMBAUGH, M. D. World distribution and historical development. In: HANSON, A. A.; BARNES, D. K.; HILL, R. R. (Eds.). Alfalfa and Alfalfa Improvement. Agronomy Monograph 29. Madison, WI: ASA-CSSA-SSSA, 1988. p.25-91. doi:10.2134/agronmonogr29.c2

MOREIRA, A.; CARVALHO, J. G. de; MORAES L. A. C.; SALVADOR, J. O. Efeito da relação cálcio e magnésio do corretivo sobre micronutrientes na alfafa. Pesquisa Agropecuária Brasileira, v.35, n.10, p.2051-2056, 2000.

MOREIRA, A.; FAGERIA, N. K.; GARCIA Y GARCIA, A. Effect of liming on the nutritional conditions and yield of alfalfa grown in tropical conditions. Journal of Plant Nutrition, v.34, n.8, p.1107-1119, 2011. DOI: https://doi.org/10.1080/01904167.2011.558155

MOREIRA, A.; HEINRICHS, R.; FREITAS, A. R. Relação fósforo e magnésio na fertilidade do solo, no estado nutricional e na produção da alfafa. Revista Brasileira de Zootecnia, v.37, n.6, p.984-989, 2008. Doi: http://dx.doi.org/10.1590/S1516-35982008000600005

MULLER, M. H.; PROSPERI, J. M.; SANTONI, S.; RONFORT, J. Inferences from mitochondrial DNA patterns on the domestication history of alfalfa (*Medicago sativa*). Molecular Ecology, v.12, p.2187-2199, 2003. doi: 10.1046/j.1365-294X.2003. 01897.x

OLIVEIRA, E.L.; PARRA, M.S. Resposta do feijoeiro a relações variáveis entre cálcio e magnésio na capacidade de troca cátions de Latossolos. Revista Brasileira de Ciência do Solo, v.27, n.5, p.859-866, 2003. Doi: http://dx.doi.org/10.1590/S0100-06832003000500010

OLIVEIRA, I. P.; ASHER, C. J.; EDWARDS, D. G.; SANTOS, R. S. M. Magnesium sulphate and the development of the common bean cultivated in an Ultisol of Northeast Australia. Scientia Agricola. v.57, n.1, p.153-157 2000. Doi: http://dx.doi.org/10.1590/S0103-90162000000100025

PROSPERI, J. M.; JENCZEWSKI, E.; MULLER, M. H.; FOURTIER, S.; SAMPOUX, J. P.; RONFORT, J. Alfalfa domestication history, genetic diversity and genetic resources. Legume Perspectives, v.4, p.13-14, 2014.

RASSINI, J. B.; FERREIRA, R. P.; CAMARGO, A. C. de. Cultivo e Estabelecimento da Alfafa. In: EMBRAPA. Cultivo e utilização da Alfafa nos trópicos. 1ª ed. São Carlos, SP: Embrapa Pecuária Sudeste, 2008. p. 37 – 52.

RICE, W. A.; PENNEY, D. C.; NYBORG, M. Effects of soil acidity on rhizobia numbers, nodulation and nitrogen fixation by alfalfa and red clover. Canadian Journal of Soil Science, v.57, n.2, p.197-203, 1977. Doi: https://doi.org/10.4141/cjss77-024

ROBSON, A. D.; PITMAN, M. G. Interactions between nutrients in higher plants. In: LÄUCHLI, A.; BIELESKI, R. L. (Eds.). Inorganic plant nutrition. Encyclopedia of Plant Physiology, v.15. Springer, Berlin, Heidelberg, 1983. p.147-180. Doi: https://doi.org/10.1007/978-3-642-68885-0_6

RODRIGUES, A. A.; NETTO, D. P.; FERREIRA, R. P.; PEDROSO, A. F.; ESTEVES, S. N. Alfafa em pastejo como parte da dieta de vacas leiteiras. São Carlos: Embrapa Pecuária Sudeste. Boletim de Pesquisa e Desenvolvimento, v.20, 2009. 24p.

SELIGA, H. The role of copper in nitrogen fixation in *Lupinus luteus* L. Plant and Soil, v.155, p.349-352, 1993. Doi: https://doi.org/10.1007/BF00025054

SILVA, J. J. R. F.; WILLIAMS, R. J. P. The uptake of elements by biological systems. Biochemistry. Part of the Structure and Bonding book series, v.29, p.67-121, 1976. Doi: https://doi.org/10.1007/BFb0116519

TRÄNKNER, M.; TAVAKOL, E.; JÁKLI, B. Functioning of potassium and magnesium in photosynthesis, photosynthate translocation and photoprotection. Physiologia Plantarum, v.163, p.414-431, 2018. Doi: https://doi.org/10.1111/ppl.12747

TROEH, F. R.; THOMPSON, L. M. Solos e fertilidade do solo. 6. ed. DOURADO NETO, D.; DOURADO, M. N. (Tradutores). São Paulo: ANDREI, 2007. 718p.